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Short communication

Molecular epidemiology of *Mycobacterium tuberculosis* strains from prison populations in Santa Catarina, Southern Brazil

Taiane Freitas Medeiros[a], Christiane Lourenço Nogueira[a], Rodrigo Ivan Prim[a], Mara Cristina Scheffer[a], Eduardo Venâncio Alves[a], Darcita Büerger Rovaris[b], Thierry Zozio[c], Nalin Rastogi[c,*], Maria Luiza Bazzo[a,**]

[a] Universidade Federal de Santa Catarina – UFSC, Campus Universitário, s/n., Florianópolis, Santa Catarina, Brazil
[b] Laboratório Central do Estado de Santa Catarina (LACEN/SC), Florianópolis, Santa Catarina, Brazil
[c] WHO Supranational TB Reference Laboratory, Institut Pasteur de la Guadeloupe, Abymes, Guadeloupe, France

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ABSTRACT

The Tuberculosis (TB) notification rates are 5 to 81 times higher in prisons worldwide when compared to the general population. The state of Santa Catarina (SC) has few epidemiological data regarding TB in prisons. The aim of this study was to evaluate the molecular epidemiology of circulating strains in prisons of SC. The study comprised 95 clinical samples from six prisons. Among the cases included, all subjects were male, predominantly caucasians, and young adults, with low education level. The positive smear in the TB diagnosis comprised 62.0% of cases. About 50% of subjects had some condition associated with TB. The Spoligotyping results showed that the most frequent lineages were LAM (50.7%), T (22.2%) and S (11.6%). The 12-loci MIRU generated 62 different genotypes. The MSTs showed evolutionary relationships between *Mycobacterium tuberculosis* spoligotypes from SC and evolutionary relationships between the prison isolates and studied parameters. This first study on TB in prison units of SC highlighted the predominance of SIT216/LAM5, and SIT34/S. Interestingly, his profile was found to be different from that observed in a previous study performed with the state's general population. This data shows the need for continued surveillance of episodes of TB occurring among prison inmates in an emerging country like Brazil.

1. Introduction

Tuberculosis (TB) is one of the world's deadliest infectious diseases. TB notification rates are 5 to 81 times higher in prisons worldwide when compared to the general population (WHO, 2014). In Santa Catarina (SC), Southern Brazil, the incidence rate within the prison system in 2014 was 1295/100,000 inhabitants, 25 times higher when compared to the general population (Brasil, 2014; Brasil, 2016). The SC prison system's capacity is sufficient to accommodate only 60% of the total inmate population, demonstrating the rate of overcrowding in SC prisons (Brasil, 2014).The prolonged stay in enclosed and poorly ventilated spaces, overcrowding, lack of control measures for air-transmitted diseases, and high HIV infection rates among prisoners encourage the TB transmission (Dara et al., 2015).

Molecular epidemiology studies are useful tools in the identification of factors of TB distribution and transmission among the inmates. In this study, Spoligotyping and Mycobacterial Interspersed Repetitive Units – Variable Number Tandem Repeat (MIRU-VNTR) analyses were employed to determine the frequency of *Mycobacterium tuberculosis* (MTB) genotypes in clinical isolates from inmates of SC prisons. The genotypes were then related to clinical and epidemiological data, and compared with non-prison TB isolates in SC to verify any prison-related specificities.

2. Materials and methods

This study was approved by the Research Ethics Committee of the Federal University of Santa Catarina under number 03448612.9.0000.0121.

Ninety five MTB clinical isolates were obtained from the Central Laboratory of Public Health of SC (LACEN-SC), from January/2010 to December/2014. Samples were collected from 95 inmates from six

* Correspondence to: N. Rastogi, WHO Supranational TB Reference Laboratory, Tuberculosis & Mycobacteria Unit, Institut Pasteur de la Guadeloupe, Morne Joliviere, BP 484, 97183 Abymes, Guadeloupe, France.
** Correspondence to: M.L. Bazzo, Department of Clinical Analysis, Federal University of Santa Catarina, Campus Universitário, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil.
E-mail addresses: rodrigo.prim@posgrad.ufsc.br (R.I. Prim), tzozio@pasteur-guadeloupe.fr (T. Zozio), nrastogi@pasteur-guadeloupe.fr (N. Rastogi), m.l.bazzo@ufsc.br (M.L. Bazzo).

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prison units in SC: State Prison Complex - COPE (São Pedro de Alcântara), Male Prison of Florianópolis - PMF, Male Prison of Tubarão - PMT, Southern Penitentiary – PS (Criciúma), Santa Augusta Prison – PSA (Criciúma) and Prison Complex of the Vale do Itajaí – CPVI (Itajaí). Drug susceptibility testing (DST) was performed with the Bactec MGIT 960. Clinical-epidemiological data on the participants were obtained from the Notifiable Diseases Information System (SINAN).

MTB DNA was extracted using cetyltrimethylammonium bromide, as described by Van Soolingen et al. (1994), followed by Spoligotyping (Kamerbeek et al., 1997) and 12-loci MIRU-VNTRs (Supply et al., 2000). For genotyping analysis and comparison with an international database, spoligotype patterns were entered in the SITVIT2 proprietary database (Couvin and Rastogi, 2014, 2015). In this database, Spoligotype International Type (SIT) and a 12-loci MIRU International Type (12MIT) designate patterns shared by two or more isolates, whereas "orphan" designates patterns reported for a single isolate.

The Pearson Chi-Square test and Fisher's exact test were employed using the website "openepi.com" to compare the results between this and other studies. p Values < 0.05 were considered statistically significant. Data on prison isolates were compared with non-prison TB isolates in SC (Nogueira et al., 2016). Lastly, evolutionary relationships were studied through the elaboration of Minimum Spanning Trees (MSTs) using the BioNumerics Software version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium).

3. Results and discussion

SC has a population of approximately 7,006,200 inhabitants, which corresponds to 3.4% of the Brazilian population (IBGE, 2017). The state's prison population corresponds to 0.25% of the SC general population and represents approximately 2.4% of the Brazilian prison population. It is estimated that over the past ten years the number of inmates in SC prisons increased > 40%, over twice the capacity of the state's prison units. Currently, the prison system's capacity is sufficient to accommodate only 60% of the total inmate population, demonstrating the rate of overcrowding that occurs in SC prisons (Brasil, 2014). SC has 49 prison units. The population of the six prisons included in this study represents 30% of the SC prison population (CNJ, 2017).

3.1. Clinical-epidemiological and demographic characteristics

Clinical and epidemiological data for the 95 prison isolates are summarized in Supplemental Table S1, Cases distribution according to prison unit was as follows: 22.1% (21/95) were from COPE, 27.4% (26/95) from PMF, 10.5% (10/95) from PMT, 2.1% (2/95) from PS, 14.7% (14/95) from PSA and 23.2% (22/95) from CPVI. Of the 95 cases, 14.7% (14/95) had not been included in the notification system; therefore, the only available data on these cases came from LACEN-SC, including gender, age, prison unit, and DST results.

Results of the smear test at the time of diagnosis were positive in 62.1% (59/95). In addition, treatment follow-up smear testing showed that 62.0% of the cases remained positive after the second month of treatment. Moreover, in more than half of the prison units, smear test conversion occurred only in the fourth month of treatment. This demonstrated that the transmission chain of TB is maintained inside the prison for many months, a concerning fact, as the risk of transmission in institutionalized interactions is 10 times higher than in casual interactions (WHO, 2014). Approximately 64% (61/95) of the individuals were tested for HIV, and 32.8% (20/61) had positive results, rate higher than that found in other prisons throughout Brazil ($p < 0.001$) (Silveira et al., 2007; Kuhleis et al., 2012). DST results showed that 9.5% (9/95) of the isolates displayed some form of drug resistance and 3.2% (3/95) were MDR-TB. Similar results were obtained in the general population with TB in SC ($p > 0.050$) (Nogueira et al., 2016). Most cases were registered as new cases (76.6%; 62/81) and 17 (21.0%) as relapse. Regarding the outcome, most cases were successfully treated (75.3%, 61/81), while 11 (13.6%) inmates abandoned treatment. Treatment default was observed mainly in younger individuals, who were HIV-positive, and had started the directly observed treatment (DOT) in prison. HIV-positive individuals have a lower chance of cure. Moreover, this default rate may be related to the interruption of treatment due to prison transfer or release. Treatment default was also related to TB/HIV coinfection ($p = 0.001$), being possibly due to the intensified adverse effects that occur with the concomitant treatment of both diseases.

3.2. Genotyping, database comparison and evolutionary relationships

Detailed information on Spoligotyping and 12-loci MIRU typing results in conjunction with demographic and epidemiological information is summarized in Supplementary Table S1. Briefly, spoligotyping identified 33 different profiles: 89 isolates were classified as belonging to 27 different SITs and six isolates were classified as orphans. There were 17 isolates with unique standards and 78 were grouped into clusters. This study resulted in five new SITs. The most frequently lineage was the Latin-American & Mediterranean (LAM), comprising 50.7% (48/95) of the isolates, followed by the T-superfamily with 22.2% (21/95), S lineage (11/95; 11.6%), East African-Indian (EAI) (4/95; 4.2%), Haarlem (3/95; 3.2%), Ural (1/95; 1.1%), and the European X lineage (1/95; 1.1%). The most frequent SITs were SIT216/LAM5 (13/95; 13.7%), SIT34/S (11/95; 11.6%) and SIT42/LAM9 (8/95; 8.4%). The other identified SITs comprised from one to six isolates each (Supplementary Table S1). The detailed analysis of spoligotyping defined clusters (representing 2 or more strains) and corresponding SITs among prison isolates in SC as compared to their global distribution in SITVIT2 database is in the Table 1. As can be easily seen, nearly all clusters corresponded to MTB Complex shared-types and lineages commonly found in South America, and specifically Brazil. The 12-loci MIRU analysis generated 62 different genotypes. Nineteen genetic groups were formed with 100% similarity, 14 of them comprising two isolates each (Supplementary Table S1), three with three isolates each (MIT34, MIT1757, MIT1761), one with seven isolates (MIT1758), and one with eight isolates (MIT1764).

To evaluate the evolutionary relationships among MTB spoligotypes in SC, MSTs were drawn for 481 isolates from inmates (data from this study) and non-inmates (Nogueira et al., 2016) (Fig. 1). A super representation of the S lineage was observed among the inmates (11.6%) when compared to the non-prison population (1.6%; $p < 0.01$). In the general population, isolates of the S lineage were observed only in the Southern region of SC (Nogueira et al., 2016), which explains its prevalence in the Criciúma prison unit, located in this region. However, the presence of SIT34/S was also verified in the unit of Great Florianópolis, demonstrating the possible dissemination of strains due to inmates transfer among SC prisons. A sub-representation of the Haarlem family was observed among the prison population (3.2%) compared to the non-prison population (12.7%; $p < 0.01$), this difference was not related to clinical-epidemiological data.

In global population of SC, EAI5 lineage representing 2.7% (SIT3099/EAI5, n = 13/481) of strains, split as 4.2% in prison (n = 4/95) vs. 2.3% (n = 9/385) in the non-prison population. In this context, it should be noted that in the global spoligotyping-based MST drawn for the 481 strains from SC, two major branches were formed, the first one mainly by the LAM lineage and the second one by the T and Haarlem lineages, corroborating earlier observations (Gomes et al., 2012). As mentioned recently (Nogueira et al., 2016), SIT3099 which was classified as EAI5 in our study; was closely linked to SIT42/LAM9 in the MST (Fig. 1B), surrounded by other major SITs from the LAM lineage (SIT64/LAM6, SIT93/LAM5, SIT216/LAM5 and SIT17/LAM2).

Considering the existing doubts about the EAI5 assignment to SIT3099 by the SITVIT2 database (Couvin and Rastogi, 2014, 2015), we decided to reevaluate its lineage based on combined spoligotyping and MIRU data

Table 1
Spoligotyping defined clusters and corresponding SITs among prison isolates of *M. tuberculosis* in Santa Catarina (representing 2 or more strains) as compared to their global distribution in SITVIT2 database.

SIT (lineage) spoligotype description	Nb (%) in study	% in study vs. SITVIT2[a]	Distribution in regions with > = 3% of a given SIT[b]	Distribution in countries with > = 3% of a given SIT[c]
SIT216 (LAM5)	13 (13.68)	**23.64**	**AMER-S 87.27**, AMER-N 9.09, EURO-S 3.64	**BRA 83.64**, USA 9.09, ITA 3.64
SIT34 (S)	11 (11.58)	1.22	AMER-N 23.11, AFRI-S 17.11, **AMER-S 14.11**, Euro-S 14.11, EURO-W 8.89, AFRI-N 4.0, CARIB 3.67, EURO-E 3.44, ASIA-W 3.33, EURO-N 3.11	ZAF 17.11, USA 14.89, ITA 11.78, CAN 8.22, **BRA 8.0**, FRA 4.0, BGR 3.44
SIT42 (LAM9)	8 (8.42)	0.21	**AMER-S 35.06**, AMER-N 10.62, EURO-S 10.14, EURO-W 8.48, AFRI-N 7.68, EURO-N 4.39, CARIB 3.8, AFRI-E 3.48, AMER-C 3.18	**BRA 14.47**, USA 10.62, COL 7.65, MAR 6.31, ITA 5.86, FRA 4.55, ARG 3.53, PER 3.32, ESP 3.0
SIT53 (T1)	6 (6.32)	0.09	**AMER-S 17.09**, EURO-W 14.15, AMER-N 12.19, EURO-S 8.51, ASIA-W 7.98, EURO-N 6.77, AFRI-E 4.65, AFRI-S 4.49, ASIA-E 3.86, AFRI-N 3.18	USA 11.96, FRA 7.14, **BRA 5.87**, ITA 4.82, TUR 4.51, ZAF 4.39, PER 4.29, AUT 3.1
SIT17 (LAM2)	5 (5.26)	0.67	**AMER-S 59.97**, AMER-N 15.93, CARIB 11.91, EURO-S 4.95	**BRA 30.52**, VEN 23.96, USA 15.93, HTI 7.36, ESP 3.88
SIT73 (T)	5 (5.26)	1.59	**AMER-S 26.98**, EURO-S 13.97, EURO-W 11.43, AMER-N 11.43, AFRI-S 8.89, AFRI-E 6.35, ASIA-E 3.81, AMER-C 3.17	**BRA 18.41**, ITA 11.75, USA 11.43, ZAF 8.89, FRA 6.35, ARG 4.44, CHN 3.81, MOZ 3.17
SIT60 (LAM4)	4 (4.21)	0.87	AFRI-W 35.36, **AMER-S 26.9**, EURO-W 6.72, AFRI-N 6.51, AFRI-W 5.86, EURO-S 5.21, AMER-N 4.12	ZAF 35.36, **BRA 15.84**, FRA 4.77, USA 4.12, MAR 4.12, GMB 3.69, ITA 3.47, VEN 3.25, PER 3.04
SIT65 (T1)	4 (4.21)	2.86	**AMER-S 40.71**, CARIB 23.57, EURO-W 12.14, AMER-N 9.29, EURO-S 4.29	**BRA 39.29**, HTI 21.43, USA 9.29, FRA 6.43, ESP 3.57
SIT3099 (EAI5)	4 (4.21)	**22.22**	**AMER-S 94.44**, ASIA-E 5.56	**BRA 94.44**, CHN 5.56
SIT64 (LAM6)	23 (5.67)	0.65	**AMER-S 58.26**, AMER-N 20.43, EURO-W 5.22, EURO-S 3.91	**BRA 44.35**, USA 20.43, GUF 5.0, PRT 3.04
SIT177 (LAM9)	3 (3.16)	2.33	**AMER-S 68.99**, EURO-S 10.85, EURO-W 7.75, AFRI-N, 3.88	**BRA 51.16**, ARG 9.3, FRA 7.75, ESP 6.98, ITA 3.88, PER 3.1
SIT613 (T1)	3 (3.16)	**15.0**	EURO-S 35.0, AMER-N 25.0, EURO-W 20.0, **AMER-S 15.0**, EURO-N 5.0	ALB 30.0, USA 25.0, **BRA 15.0**, AUT 15.0, ITA 5.0, SWE 5.0, FRA 5.0
4004 (Unknown)	3 (3.16)	**100**	**AMER-S 100.0**	**BRA 100.0**
93 (LAM5)	2 (2.11)	0.42	**AMER-S 53.88**, CARIB 15.72, AMER-N 15.72, EURO-S 6.71, EURO-W 3.77	VEN 19.71, USA 15.72, **BRA 14.47**, PER 13.42, HTI 10.06, ITA 4.4, GLP 3.98
729 (LAM1)	2 (2.11)	**15.38**	**AMER-S 53.85**, CARIB 38.46, AFRI-S 7.69	BRA 53.85, HTI 30.77, GLP 7.69, ZAF 7.69
2263 (LAM9)	2 (2.11)	**16.67**	AMER-S 50.0, AMER-N 20.0, EURO-S 10.0, EURO-N 10.0, ASIA-N 10.0	BRA 50.0, USA 20.0, RUS 10.0, ITA 10.0, FIN 10.0

[a] Note that values above 12.5% (percentage in study vs. SITVIT2) are shown in **bold**.

[b] Worldwide distribution is reported for regions with > 3% of a given SITs as compared to their total number in the SITVIT2 database. The definition of macro-geographical regions and sub-regions (http://unstats.un.org/unsd/methods/m49/m49regin.htm) is according to the United Nations; Regions: AFRI (Africa), AMER (Americas), ASIA (Asia), EURO (Europe), and OCE (Oceania), subdivided in: E (Eastern), M (Middle), C (Central), N (Northern), S (Southern), SE (South-Eastern), and W (Western). Furthermore, CARIB (Caribbean) belongs to Americas, while Oceania is subdivided in 4 sub-regions, AUST (Australasia), MEL (Melanesia), MIC (Micronesia), and POLY (Polynesia). Note that in our classification scheme, Russia has been attributed a new sub-region by itself (Northern Asia) instead of including it among rest of the Eastern Europe. It reflects its geographical localization as well as due to the similarity of specific TB genotypes circulating in Russia (a majority of Beijing genotypes) with those prevalent in Central, Eastern and South-Eastern Asia. Note that values for AMER-S are shown in **bold**.

[c] The country codes are according to http://www.worldatlas.com/aatlas/ctycodes.htm; countrywide distribution is only shown for SITs with ≥ 3% of a given SITs as compared to their total number in the SITVIT2 database. Note that values for Brazil are shown in **bold**.

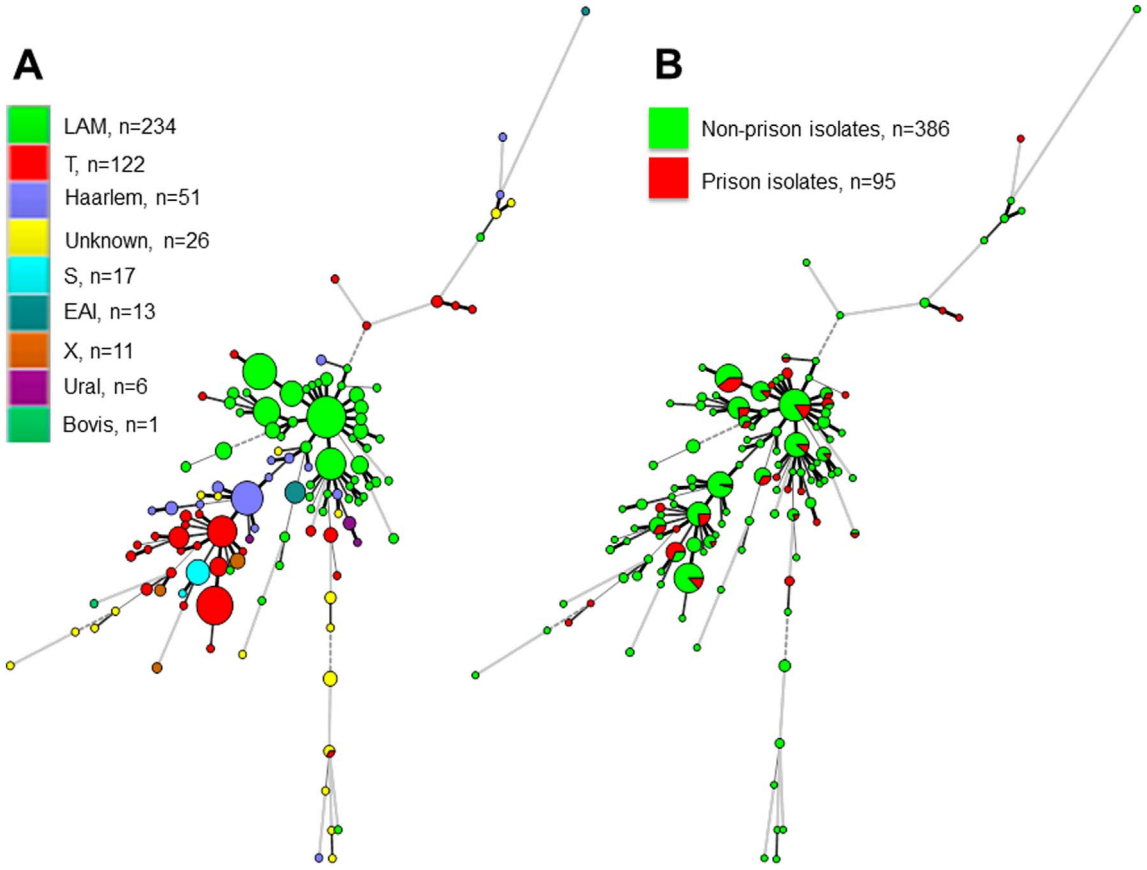

Fig. 1. Minimum spanning tree (MST) illustrating evolutionary relationships between *M. tuberculosis* spoligotypes from Santa Catarina, Brazil (n = 481 strains split as 385 non-prison vs. 95 prison isolates); (A). The MST with colors of the circles indicating the lineages to which each specific pattern belongs to; (B). Same MST showing the distribution of prison (n = 95) vs. non-prison (n = 385) isolates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

available and its geographical distribution using the in-house SITVITEXTEND database, and tools SPOTCLUST (http://tbinsight.cs.rpi.edu/run_spotclust.html) and TB-Lineage (http://tbinsight.cs.rpi.edu/run_tb_lineage.html). Depending on the algorithm chosen the SIT3099 binary pattern (11111111111111111111000011111100000000011111) was classified as LAM9 with a probability of 0.998 (CBN or KBBN). Considering that in an independent investigation (results not shown), the SIT3099 strains were found to be associated with the RD-Rio deletion (which is specific of LAM strains; (Lazzarini et al., 2007)); we decided to revise SIT3099 from EAI5 to LAM9. Our decision was further corroborated by the values observed for MIRU locus 24 (MLVA 2687, highlighted in **bold**) for the 4 SIT3099 isolates in our study: 222427**1**52332, 224126**1**62631, 224425**1**52633, and 222326**1**41331; indeed a value of 1 for MIRU24 designates Euro-American Lineage of recent evolution (to which LAM belongs to), while a value above 1 is characteristic of ancestral isolates such as the EAI (Supply et al., 2006). Subsequently, this information was provided to SITVIT database curator, leading to the official change of SIT3099/EAI5 to SIT3099/LAM9 on June 12th, 2017 (http://www.pasteur-guadeloupe.fr:8081/SpolSimilaritySearch/; (Couvin et al., 2017)) (Fig. 2).

Aware of the advantages of the 15- or 24-loci MIRU formats over the 12-loci format, particularly when MIRU-VNTRs are used as the sole typing method (Jagielski et al., 2016). Nevertheless, a combination of spoligotyping and 12-loci MIRUs has been successfully used in resources-limited settings to decipher "potential" transmission chains (Couvin and Rastogi, 2014; Jagielski et al., 2016). Combining the 12-loci MIRUs with spoligotyping allowed to assess ongoing transmission, underlining a total of 12 clusters containing 35 isolates (Supplemental Table S1), which significant considering the relatively small sample size. In conjunction to the present cluster analysis, MST analysis further confirmed the unique nature of the large clusters comprised of LAM (SIT216/LAM5/MIT1764 n = 8; SIT177/LAM9/MIT1756 n = 3; SIT60/LAM4/MIT1761 n = 3, followed by SIT216/LAM5/MIT1760 n = 2; note that a larger group contained isolates from all prison units while a smaller group corresponded to isolates from Itajaí); and S lineages clusters from the prison units of Criciúma, Florianópolis, and São Pedro de Alcântara (SIT34/S/MIT1758 n = 7, and S34/S/MIT1766 n = 2). The epidemiological variables, age group, HIV serology, case input registration and smear test results were proportionally distributed in various groups according to the general distribution; and these variables did not show any significant relationship with the phylogenetic groups within specific prison units. Nevertheless, all S lineage strains remained within the same MST group; hence considering that the combination of spoligotyping and 12-loci MIRUs has a fairly good discriminatory powers for genomic analysis of MTB (Pitondo-Silva et al., 2013), our results not only highlight the very high similarity between S lineage strains circulating in different prison units, but further reinforce the possibility of intra- and inter-institutional dissemination.

In conclusion, this first study on TB in prison units in the state of Santa Catarina highlighted the predominance of SIT216/LAM5 (almost exclusively in one of the prison units), and SIT34/S, which in the majority was from the southern region of the state, having been detected also in the region of Great Florianópolis. Interestingly, his profile was found to be different from that observed in a previous study performed with the state's general population. This data is important for the development of specific policies and strategies for TB control in the prison population, and shows the need for continued surveillance of episodes of TB occurring among prison inmates in an emerging country like Brazil.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2017.12.010.

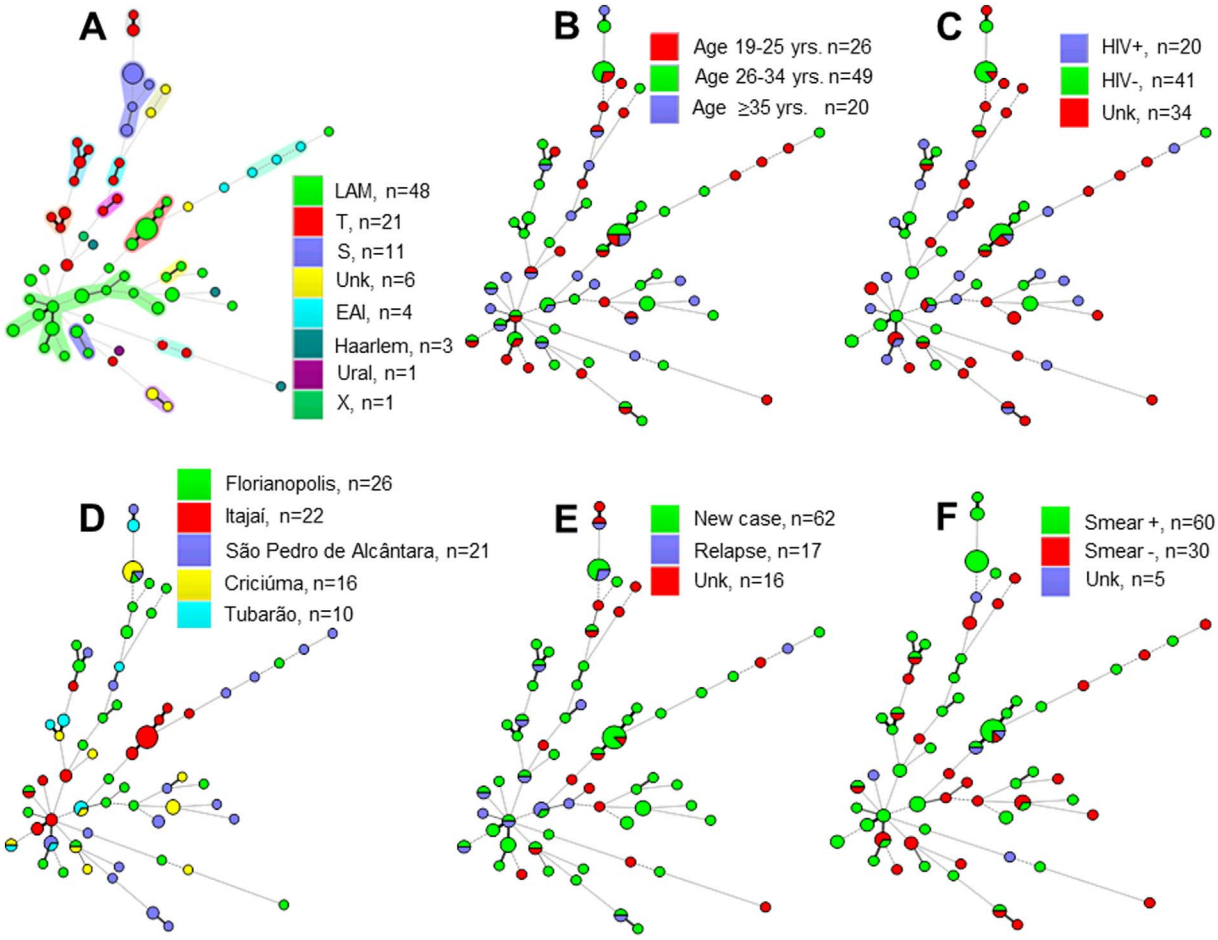

Fig. 2. Minimum spanning trees (MST) illustrating evolutionary relationships between the *M. tuberculosis* strains isolated from the prison inmates (n = 95 strains) genotyped using spoligotyping and 12-loci MIRUs. The individual trees show the relationships between the genotypes in function of studied parameters: (A) Genotypic lineages; (B) Age groups; (C) HIV serology; (D) City of origin; (E) New vs. relapse case; (F) Smear positive vs. negative samples. The structure of the tree represented by branches (continuous vs. dashed and dotted lines) and circles (representing each individual pattern) is the same as in Fig. 1.

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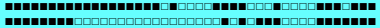
Supplemental Table S1. A summary of Spoligotyping and 12-loci MIRU typing results, and available demographic and epidemiological information on the 95 M. tuberculosis isolates from prison inmates in Santa Catarina, Brazil.

Strain	Year	Sex	Age	City of Isolation (GeoName ID)	Country of Origin	Spoligotype Description	Octal code	Lineage	SIT	MIRU12	12-MIT	DST Code	HIV Serology	Remarks
17892	2013	M	24	Tubarão (3445993)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	LAM9	42	223226143321	10	1	negative	Smear positive - new case - Case outcome:dropout
109048	2013	M	33	Criciúma (3465197)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	LAM9	42	223226143321	10	1	negative	Smear positive - relapse case - Case outcome:cure
94787	2014	M	30	Tubarão (3445993)	BR	■■□■■■■■■■■■□■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	677737607760771	LAM2	17	224325153324	34	1	negative	Smear positive - new case -
78804	2010	M	31	Criciúma (3465197)	BR	■■□■■■■■■■■■□■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	677737607760771	LAM2	17	224325153324	34	1		Smear test-positive-relapse case-cure
58566	2011	M	49	Tubarão (3445993)	BR	■■□■■■■■■■■■□■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	677737607760771	LAM2	17	224325153324	34	1	positive	Smear test-positive-relapse case-dropout
109886	2014	M	32	Florianopolis (3463237)		■■■■■■■■■■■■■■■■■□■■■■■■■■■■■■■■□□□□■■■■■■■	777776777760771	X1	119	224325143324	117	3		Smear negative
65102	2012	M	29	Itajaí (3460845)	BR	■■■■■□■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	767777607760771	LAM9	2263	224126152321	163	2		Smear positive - relapse case - Case outcome:dropout
10549	2014	M	40	Florianopolis (3463237)	BR	■■■■■□■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	767777607760771	LAM9	2263	224126152321	163	1		Smear negative - new case - Case outcome:cure
44047	2013	M	26	São Pedro de Alcântara (3448344)	BR	■■■■■■■■□□□■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	776177607760771	LAM3	33	224226153314	186	1	negative	Smear positive - new case - Case outcome:cure
61846	2012	M	24	Itajaí (3460845)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	LAM9	42	124326153324	246	1	negative	Smear positive - new case - Case outcome:cure
31034	2011	M	27	Itajaí (3460845)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	LAM9	42	124326153324	246	2	negative	Smear test-positive-return after dropout-cure
55224	2013	M	27	Itajaí (3460845)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	LAM9	42	123326153324	323	1	negative	Smear positive - new case - Case outcome:cure
58987	2013	M	42	Itajaí (3460845)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	LAM9	42	123326153324	323	1	negative	Smear positive - new case - Case outcome:cure
9718	2011	M	42	Florianopolis (3463237)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	LAM9	42	224326173424	347	2	positive	Smear test-positive-return after dropout-cure
88543	2014	M	41	Florianopolis (3463237)		■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	225325153533	471	3	positive	Smear negative - transference -
24605	2012	M	39	Florianopolis (3463237)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■□■■■□□□□■■■■■■■	777777607560771	LAM6	64	125326133222	812	1	negative	Smear negative
95140	2013	M	31	Criciúma (3465197)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■□■■■□□□□■■■■■■■	777777607560771	LAM6	64	125326133222	812	1		Smear negative - new case -
59256	2010	M	22	São Pedro de Alcântara (3448344)	BR	■■■■■■■■■■■■□■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777737607760771	LAM5	93	224227153321	862	1		Smear test-positive-new case-cure
47313	2011	M	36	São Pedro de Alcântara (3448344)	BR	■■■■■■■■■■■■□■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777737607760771	LAM5	93	224227153321	862	1		Smear test-positive-new case-cure
70899	2010	M	28	São Pedro de Alcântara (3448344)	BR	■■■■■■■■■■■■■■■■■■■■□□□□□□□□□□□□□□□□□■■□■■■	777777600000331	Unknown	4004	211426152223	1751	1		Smear test-positive-relapse case-cure
70904	2010	M	20	São Pedro de Alcântara (3448344)	BR	■■■■■■■■■■■■■■■■■■■■□□□□□□□□□□□□□□□□□■■□■■■	777777600000331	Unknown	4004	211426152223	1751	1	positive	Smear test-negative-new case-dropout
45426	2012	M	34	Florianopolis (3463237)	BR	■■□■■■■■■■■■□■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	677737607760771	LAM2	17	223425153321	1756	1	positive	Smear negative - return after dropout - Case outcome:cure
108555	2012	M	29	Criciúma (3465197)	BR	□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	377777607760771	LAM9	177	223425162331	1757	1	negative	Smear negative - new case - Case outcome:cure
61517	2012	M	30	Criciúma (3465197)	BR	□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	377777607760771	LAM9	177	223425162331	1757	1	negative	Smear positive - new case - Case outcome:cure
118442	2013	M	31	Criciúma (3465197)	BR	□■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	377777607760771	LAM9	177	223425162331	1757	1	negative	Smear negative - new case - Case outcome:cure
55961	2012	M	29	Criciúma (3465197)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	231526142332	1758	1	negative	Smear positive - new case - Case outcome:cure
110452	2013	M	26	Criciúma (3465197)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	231526142332	1758	1	negative	Smear positive - new case - Case outcome:cure
90490	2013	M	25	Florianopolis (3463237)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	231526142332	1758	1	negative	Smear positive - new case -
123660	2013	M	25	Criciúma (3465197)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	231526142332	1758	1	negative	Smear positive - new case - Case outcome:cure
39941	2013	M	28	São Pedro de Alcântara (3448344)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	231526142332	1758	1		Smear positive - relapse case - Case outcome:cure
109637	2014	M	27	Criciúma (3465197)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	231526142332	1758	1	negative	Smear positive - new case -
58108	2010	M	34	Criciúma (3465197)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	231526142332	1758	1	negative	Smear test-positive-relapse case-cure
65096	2012	M	45	Itajaí (3460845)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777777777760771	T1	53	224316153224	1759	1	negative	Smear positive - relapse case - Case outcome:cure
86845	2012	M	23	Itajaí (3460845)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777777777760771	T1	53	224316153224	1759	1	negative	Smear positive - new case - Case outcome:cure
98391	2012	M	28	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151333	1760	1	negative	Smear positive - new case - Case outcome:cure
18510	2012	M	20	Itajaí (3460845)		■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151333	1760	1		
44931	2012	M	25	São Pedro de Alcântara (3448344)		■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■□■■■	777777607760731	LAM4	60	124426153324	1761	1		Smear negative - new case - Case outcome:cure
77693	2013	M	27	Tubarão (3445993)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■□■■■	777777607760731	LAM4	60	124426153324	1761	2	positive	Smear negative - new case - Case outcome:dropout
31699	2013	M	31	São Pedro de Alcântara (3448344)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■□■■■	777777607760731	LAM4	60	124426153324	1761	3		Smear positive - new case - Case outcome:cure
63831	2012	M	27	Tubarão (3445993)	BR	■■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777177777760771	T1	613	230525141322	1762	1	negative	Smear positive - transference - Case outcome:cure
97507	2013	M	27	Tubarão (3445993)	BR	■■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777177777760771	T1	613	230525141322	1762	1	negative	Smear positive - relapse case - Case outcome:cure
124879	2013	M	26	Tubarão (3445993)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777777777760771	T1	53	223325163635	1763	1	negative	Smear negative - new case - Case outcome:cure
68556	2010	M	26	Tubarão (3445993)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777777777760771	T1	53	223325163635	1763	1	negative	Smear test-positive-relapse case-cure
3491	2012	M	29	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151331	1764	1		Smear positive - new case - Case outcome:cure
311	2013	M	20	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151331	1764	1	negative	Smear positive - new case - Case outcome:cure
13438	2013	M	21	Itajaí (3460845)		■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151331	1764	1		
31643	2013	M	30	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151331	1764	1	negative	Smear positive - new case - Case outcome:cure
126766	2013	M	27	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151331	1764	1	negative	Smear positive - new case -
130581	2013	M	35	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151331	1764	1	negative	Smear positive - new case - Case outcome:cure
65512	2014	M	35	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151331	1764	1	positive	Smear negative - new case - Case outcome:cure
23999	2014	M	31	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426151331	1764	1	negative	Smear positive - new case - Case outcome:cure
56261	2013	M	53	Florianopolis (3463237)	BR	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■□□□□■■■□■■■	777737777760731	T	73	223425163221	1765	0		Smear negative - new case - Case outcome:cure
110863	2013	M	26	Florianopolis (3463237)	BR	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■□□□□■■■□■■■	777737777760731	T	73	223425163221	1765	1	negative	Smear positive - return after dropout - Case outcome:cure
91242	2014	M	37	Florianopolis (3463237)		■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	223426162531	1766	1		Smear negative
23399	2014	M	24	Florianopolis (3463237)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	223426162531	1766	1	negative	Smear negative - new case -
97566	2013	M	41	Tubarão (3445993)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□□■■■■	777777777760471	T1	65	223426162331	1767	1	positive	Smear positive - new case - Case outcome:cure
111213	2014	M	21	Florianopolis (3463237)		■■■■■■□■■■■■■■■■■■■■■■■■■■■■□□□□□□□□■■■□□□□	773777777400700	Unknown	Orphan	223426162331	1767	1		Smear negative
24009	2014	M	30	Itajaí (3460845)	BR	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■□□□□■■■□■■■	777737777760731	T	73	223325163221	1768	1	positive	Smear negative - new case - Case outcome:cure
101990	2014	M	24	Florianopolis (3463237)		□□□□□□□□□□□□□□□□□□□□□□□□■□□□□□□■□□□□■■■■■■■	000000004020771	H2	2	225313153233	Orphan	1		Smear negative
79684	2014	M	40	Florianopolis (3463237)	BR	■■□■■■■■■■■■□■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	677737607760771	LAM2	17	224426163321	Orphan	1	negative	Smear negative - transference -
11631	2014	M	19	Florianopolis (3463237)		■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	233426162334	Orphan	1		
94048	2014	M	22	Florianopolis (3463237)	BR	■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	776377777760771	S	34	233425143534	Orphan	3		Smear positive - new case -
69683	2014	M	21	Florianopolis (3463237)		■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	LAM9	42	224425153421	Orphan	1		Smear negative
10532	2011	M	24	Criciúma (3465197)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■	777777777720771	H3	50	226335153324	Orphan	1	negative	Smear test-positive-new case-cure
50612	2012	M	26	Tubarão (3445993)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777777777760771	T1	53	213325153635	Orphan	1	positive	Smear positive - new case - Case outcome:cure
9496	2012	M	32	Criciúma (3465197)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777777777760771	T1	53	223325153635	Orphan	1	negative	Smear positive - new case - Case outcome:cure
63934	2013	M	19	Florianopolis (3463237)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■□■■■	777777607760731	LAM4	60	124428153323	Orphan	3	positive	Smear positive - new case - Case outcome:cure
80412	2013	M	34	Criciúma (3465197)	BR	■■■■■■■■■■■■■■■■■■■■□□□□■■■■□■■■□□□□■■■■■■■	777777607560771	LAM6	64	123325163222	Orphan	1	negative	Smear positive - new case - Case outcome:cure
128313	2014	M	22	São Pedro de Alcântara (3448344)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□□■■■■	777777777760471	T1	65	223425162332	Orphan	1		Smear positive - new case -
21736	2011	M	26	Florianopolis (3463237)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□□■■■■	777777777760471	T1	65	224525143233	Orphan	1		Smear test-positive-return after dropout-cure
NR 36	2011	M	50	Florianopolis (3463237)	BR	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□□■■■■	777777777760471	T1	65	224525143334	Orphan	0	positive	Smear test-positive-new case-dropout
1545	2013	M	22	São Pedro de Alcântara (3448344)	BR	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■□□□□■■■□■■■	777737777760731	T	73	222425163221	Orphan	1	negative	Smear positive - new case - Case outcome:cure
NR 59	2011	M	27	Florianopolis (3463237)	BR	■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■□□□□■■■□■■■	777737777760731	T	73	223426143221	Orphan	0	positive	Smear test-positive-new case-dropout
85160	2014	M	29	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426171331	Orphan	1	negative	Smear positive - new case - Case outcome:cure
80228	2014	M	31	Itajaí (3460845)	BR	■■■■■■■■■■■■□□■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777717607760771	LAM5	216	125426171332	Orphan	1	negative	Smear positive - new case - Case outcome:cure
90197	2012	M	42	Itajaí (3460845)	BR	■■■■■■□■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	773777607760771	LAM9	492	125426143234	Orphan	1	positive	- new case - Case outcome:dropout
31709	2013	M	39	São Pedro de Alcântara (3448344)		■■■■■■■■■□□■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777177777760771	T1	613	130525141322	Orphan	1		Smear positive
36135	2012	M	26	São Pedro de Alcântara (3448344)		■■□■□■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	657777607760771	LAM1	729	224226153631	Orphan	1	negative	Smear positive - new case - Case outcome:cure
52788	2014	M	32	Criciúma (3465197)	BR	■■□■□■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	657777607760771	LAM1	729	224326152631	Orphan	1	positive	Smear positive - new case - Case outcome:cure

62930	2010	M	21	São Pedro de Alcântara (3448344)	BR		777777600560731	T2	1664	121426142222	Orphan	1		Smear test-positive-new case-dropout
14802	2011	M	29	São Pedro de Alcântara (3448344)	BR		774377607760700	LAM4	2514	222426161331	Orphan	1		Smear test-negative-new case-cure
25595	2012	M	24	São Pedro de Alcântara (3448344)			777777607700171	EAI5	3099	222427152332	Orphan	1		Smear positive - new case - Case outcome:cure
82888	2012	M	24	São Pedro de Alcântara (3448344)	BR		777777607700171	EAI5	3099	224126162631	Orphan	1	positive	Smear positive - return after dropout - Case outcome:cure
94053	2014	M	21	Florianopolis (3463237)			777777607700171	EAI5	3099	224425152633	Orphan	1		Smear negative
66171	2010	M	31	São Pedro de Alcântara (3448344)	BR		777777607700171	EAI5	3099	222326141331	Orphan	1		Smear test-negative-new case-cure
57987	2010	M	36	São Pedro de Alcântara (3448344)	BR		777761607720771	H3	4002	223425163333	Orphan	1	positive	Smear test-positive-new case-cure
68659	2010	M	31	São Pedro de Alcântara (3448344)	BR		777777600000331	Unknown	4004	111426152213	Orphan	1		Smear test-negative-new case-cure
23409	2014	M	25	Florianopolis (3463237)			777767607560731	LAM6	4131	124426152344	Orphan	3		Smear negative
111232	2014	M	27	Florianopolis (3463237)			777777777600700	Unknown	4132	223526142331	Orphan	1		Smear negative
89126	2014	M	32	Itajaí (3460845)	BR		777777607500771	Unknown	4133	125425141331	Orphan	1	positive	Smear positive - new case - Case outcome:cure
19440	2012	M	26	São Pedro de Alcântara (3448344)			777777207420731	Ural-1	Orphan	124326163532	Orphan	1		Smear negative - new case - Case outcome:cure
78421	2013	M	28	Criciúma (3465197)	BR		776000002560771	T1	Orphan	123426163234	Orphan	1	positive	Smear positive - new case - Case outcome:cure
11946	2013	M	33	São Pedro de Alcântara (3448344)	BR		773767607760171	LAM9	Orphan	224126142631	Orphan	1	negative	Smear negative - new case - Case outcome:cure
20685	2014	M	36	Florianopolis (3463237)			776000002760771	T1	Orphan	124526153234	Orphan	0		
NR 28	2011	M	37	Florianopolis (3463237)	BR		477777607560771	LAM6	Orphan	224526143331	Orphan	0	positive	Smear test-negative-new case-dropout

Newly created SITs are highlighted in yellow
Orphan spoligotypes are highlighted in blue
Newly created 12-loci MITs are highlighted in green
Orphan 12-loci MIRU patterns are highlighted in mauve